Strahilevitz describes immunoassays of psychoactive drugs (see the Abstract). The reference fails to describe apoprotein B, HbA_1C, serum amyloid A protein, or thrombin-antithrombin III complex as the antigen to be detected by the assay described therein. Moreover, there is no suggestion or motivation from Strahilevitz to detect those antigens. Accordingly, the claims are not anticipated by or obvious over these references. Withdrawal of this ground of rejection is respectfully requested.

The rejections of the claims under 35 U.S.C. §102(b) or, in the alternative, under 35 U.S.C. §103(a) over EP 617 285 (EP '285) are respectfully traversed. This reference fails to disclose or suggest the claimed immunoassay method.

EP '285 describes a method for reducing the Hook effect in immunoassays with particulate carriers (see the Title and the Abstract). The reference fails to describe apoprotein B, HbA_1C, serum amyloid A protein, or thrombin-antithrombin III complex as the antigen to be detected by the assay described therein (see page 3, fourth full paragraph). Moreover, there is no suggestion or motivation from Strahilevitz to detect those antigens. Accordingly, the claims are not anticipated by or obvious over these references. Withdrawal of this ground of rejection is respectfully requested.

The rejection of the claims under 35 U.S.C. §103(a) over Cragle et al. (WO 85/02258) in view of Strahilevitz and EP '285 is respectfully traversed. These references, taken in combination, fail to suggest the claimed immunoassay method.

Cragle et al. describe an immunoassay method (see the Abstract). The reference fails to describe apoprotein B, HbA_1C, serum amyloid A protein, or thrombin-antithrombin III complex as the antigen to be detected by the assay described therein. Moreover, there is no suggestion or motivation from Strahilevitz to detect those antigens.

As discussed above, Strahilevitz and EP '285 both fail to disclose or suggest detecting apoprotein B, HbA_1C, serum amyloid A protein, or thrombin-antithrombin III complex.

Since Cragle et al., Strahilevitz, and EP '285 all fail to suggest detecting the antigen specified in Claims 7 and 21, these references, taken in combination, fail to suggest the claimed immunoassay method. Accordingly, the claims are not obvious over these references. Withdrawal of this ground of rejection is respectfully requested.

In addition, the Applicants have conducted Supplemental Examples within the scope of the claimed method. These Supplemental Examples are attached hereto.

The rejection of the claims under 35 U.S.C. §112, second paragraph, is respectfully traversed. In Claims 7 and 21 measuring the amount of agglutinate as recited in (b) of each claim accomplishes the goal set forth in the preamble of these claims. In addition, the claims are not interpreted in a vacuum. Rather, the claims are interpreted in light of the specification, and the specification provides detailed guidance regarding how the optically measured amount of the agglutinate formed in (a) correlates with detecting the antigen. Accordingly, the claims are definite within the meaning of 35 U.S.C. §112, second paragraph. Withdrawal of this ground of rejection is respectfully requested.

Applicants submit that the present application is in condition for allowance. Early notice to this effect is earnestly solicited.

Respectfully submitted,

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IN THE CLAIMS

--7. (Twice Amended) An agglutination immunoassay for detecting an antigen in a sample, comprising:

(a) sequentially contacting the sample with

(i) a first antibody which is capable of specifically binding to a first binding site on the antigen, wherein the first antibody is immobilized on an insoluble carrier, and then

(ii) a second antibody which is capable of specifically binding to a second binding site on the antigen, wherein the second antibody is free,

thereby forming, when the antigen is present in the sample, an agglutinate comprising the first antibody, the antigen, and the second antibody; followed by

(b) optically measuring the amount of the agglutinate formed in (a),

wherein the antigen is apoprotein B, HbA_1C, serum amyloid A protein, or thrombin-antithrombin III complex

[wherein one of the antibodies has high specificity for the antigen while the other antibody does not have strict specificity for the antigen].

21. (Twice Amended) An agglutination immunoassay for detecting an antigen in a sample, comprising:

(a) sequentially contacting the sample with

(i) a first antibody which is capable of specifically binding to a first binding site on the antigen, wherein the first antibody is free, and then

(ii) a second antibody which is capable of specifically binding to a second binding site on the antigen, wherein the second antibody is immobilized on an insoluble carrier,

thereby forming, when the antigen is present in the sample, an agglutinate comprising the first antibody, the antigen, and the second antibody; followed by

(b) optically measuring the amount of the agglutinate formed in (a),

wherein the antigen is apoprotein B, HbA_1C, serum amyloid A protein, or thrombin-antithrombin III complex

[wherein one of the antibodies has high specificity for the antigen while the other antibody does not have strict specificity for the antigen].--

Please add the following claims.

--Claims 35-42 (New)--

SUPPLEMENTAL EXAMPLES

Supplemental Example 1

A sensitized latex carrier was prepared according to the same process as described in Examples of the present specification, except that an anti-malondialdehyde low density lipoplotein (MDALDL) monoclonal antibody was used as an immobilized antibody on the latex. The anti-apoprotein B antibody as described in Examples of the present invention was used as a free antibody. The results of this supplemental example are shown in Table 1.

Table 1

MDALDL*1 (μg/ml)	ML25*2 (mOD)	Invention*3 (mOD)
0.0	-1.1	-0.9
1.6	0.2	-0.3
3.1	-0.4	1.1
6.3	-2.0	1.3
12.5	-2.1	8.2
25.0	-0.8	27.8
50.0	0.2	90.8

*1: Malondialdehyde low density lipoplotein used as a sample

*2: Monoclonal antibody against MDALDL

*3: Anti-MDALDL monoclonal antibody(immobilized)+ anti-apoprotein B polyclonal antibody (free)

Discussion:

The immunoassay of this example is characterized by the combination system consisting of the anti-MDALDL monoclonal antibody immobilized on a latex carrier and the anti-apoprotein B polyclonal antibody serving as a free antibody. MDALDL

is a variant of LDL, having a structurally different part from the normal LDL in the terms that this protein is modified at a specific site of the apoprotein B of LDL. The immobilized monoclonal antibody (ML25) recognizes such a modified site of MDALDL. The absorbance change was hardly observed at this phase where the first antibody ML25 alone was used(see second column of the above table). However, the degree of absorbance was significantly improved when the anti-apoprotein B polyclonal antibody (free) was added in combination (see third column). This indicates that the immunoassay of this example, characterized by the combination use of the immobilized anti-MDALDL monoclonal antibody and the free anti-apoprotein polyclonal antibody, has the excellent capability to detect only MDALDL having the modified site of apoprotein B from within the mixture including the normal LDL.

Supplemental Example 2

A sensitized latex carrier was prepared according to the same process as described in Examples of the present specification, except that an anti-apoprotein B monoclonal antibody (16216) was used as an immobilized antibody on the latex. The anti-MDALDL antibody ML25 as described in the above Supplemental Example 1 was used as a free antibody. The preparation of the immobilized monoclonal antibody was performed by intraperitoneally culturing in a mouse and then purifying via the DEAE-Toyopearl column chromatography, according to the conventionally used method (Kotani K. et al., Biochim Biophys Acta, Nov. 17, 1994; 1215(1-2):121-5). The results of this supplemental example are shown in Table 2.

Table 2

MDA-LDL[*1] (μg/ml)	Apo B[*2] (16216) (mOD)	Invention[*3] (mOD)
0.0	-1.1	0.4
1.6	0.2	1.3
3.1	-0.4	1.8
6.3	-2.0	3.2
12.5	-2.1	8.3
25.0	-0.8	28.3
50.0	0.2	78.7
100.0	1.9	133.4

*1: Malondialdehyde low density lipoplotein used as a sample

*2: Monoclonal antibody against apoprotein B

*3: Anti-apoprotein B monoclonal antibody (immobilized) + anti-MDALDL monoclonal antibody ML25 (free)

Discussion:

The immunoassay of this example is characterized by the combination system consisting of the anti-apoprotein B monoclonal antibody immobilized on a latex carrier and the anti-MDALDL monoclonal antibody serving as a free antibody. The absorbance change was hardly observed under the condition in which the immobilized monoclonal antibody (Apo B 16216) was solely employed (see second column). However, the degree of absorbance was significantly improved when anti-MDALDL monoclonal antibody ML25 was used in combination (see third column). This indicates that the immunoassay of this example, characterized by the combination use of anti-apoprotein B monoclonal antibody and the free anti-MDALDL monoclonal antibody, has the excellent capability to detect only the modified apoprotein B from the mixture including the normal ones.

Supplemental Example 3

0.1 g of human serum albumin (Sigma) and 50 μl of sodium cyanate (500 mM; Kisida Chemicals) were dissolved into 5 ml of 0.1 M phosphate buffer (pH 8), warmed at 37°C for 2 hors, and dialyzed against a 20 mM phosphate buffer (pH 7.2)containing 150 mM NaCl, to obtain carbamylated HAS (Engbaek et al., Clin. Chem. 35, 93-97, 1989). The carbamylated HAS thus obtained was used as an immunogen in a mouse, thereby preparing monoclonal antibodies against the immunogenic cells produced by this immunization. After this process was completed, a specific monoclonal antibody that is selectively reactive with the carbamylated albumin, but not with non-human albumin, was isolated. The immunoassay of this example was performed according to the same process as already described in the present specification, except that the anti-carbamylated HAS monoclonal antibody was used as an immobilized antibody and the anti-albumin polyclonal antibody was used as a free antibody. The results of this supplemental example are shown in Table 3.

Table 3

Carbamylated HSA*1 (μg/ml)	CH46405*2 (mOD)	Invention*3 (mOD)
0.5	1.2	0.5
12.5	1.5	5.3
25.0	0.2	10.4
50.0	0.5	24.5
100.0	1.6	64.3

*1: Carbamylated HAS used as an sample

*2: Monoclonal antibody against carbamylated HAS

*3: Anti- carbamylated HAS monoclonal antibody (immobilized) + anti-HAS polyclonal antibody (free)

Discussion:

The immunoassay of this example is characterized by the combination system consisting of the anti-carbamylated modified albumin monoclonal antibody immobilized on a latex carrier and the anti-albumin polyclonal antibody serving as a free antibody. Carbamylated modified albumin is a variant of albumin, having a structurally different part from the normal albumin in the terms that its amino acid moiety is partly modified by the urea nitrogen in blood. The anti-carbamylated modified albumin monoclonal antibody recognizes such a modified site of this albumin. The absorbance change was hardly observed under the condition in which the immobilized monoclonal antibody (CH46405) was solely employed (see second column). However, the degree of absorbance is significantly improved when the anti-albumin polyclonal antibody was used in combination (see third column). This indicates that the immunoassay of this example, characterized by the combination use of the anti-carbamylated modified albumin monoclonal antibody and the anti-albumin polyclonal antibody, has the excellent capability to detect only the modified albumin from within the mixture including the normal albumin.